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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Li *et al.*	Confirmation No.: 8877
Application No.: 09/804,014	Group Art Unit: 1636
Filed: March 12, 2001	Examiner: Sullivan, Daniel M.
For: NOVEL POLYPEPTIDES AND NUCLEIC ACIDS ENCODING SAME	Attorney Docket No.: 15966-721 (Cura-221)

DECLARATION OF DR. DANIEL RIEGER UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Daniel Rieger, Ph.D., do declare and state that:

1. I held the position of Research Scientist at CuraGen Corporation during 2001– 2003, and currently hold the position of Patent Scientist at CuraGen Corporation. CuraGen Corporation is the owner of the entire right, title and interest in, to and under the invention described and claimed in the above-identified patent application.

2. I received a Ph.D. from Wayne State University in Detroit, Michigan. My academic and technical experience and honors, and a list of my publications, are set forth in my *curriculum vitae*, attached hereto as Appendix 1.

3. I have been asked to comment on the utility of the nucleic acids described and claimed in the above-identified U.S. Application No. 09/804,014 ("the '014 application"). I have read and understand the '014 application. I have also read the Office Action dated January 16, 2004, the Amendment Under 37 C.F.R. § 1.111 that was

filed on June 16, 2004 ("June 16 Amendment"), and the Office Action dated September 21, 2004. I understand that an issue relevant to the rejection under 35 U.S.C. § 112 is whether a person skilled in the art can use the claimed nucleic acids as asserted in the specification without undue experimentation. I present this declaration to address this issue.

4. It is my opinion that the instant specification enables one of ordinary skill in the art to use the claimed nucleic acids to (1) identify therapeutics that modulate the Kv channel (*i.e.*, the function of the polypeptide of SEQ ID NO: 8), and thereby treat disorders such as diabetes or neuromuscular disorders; and (2) differentiate skeletal muscle from other tissue types.

5. The instant specification teaches that NOV4 (polypeptide having the amino acid sequence of SEQ ID NO: 8) is a new member of a subclass of Kv channels that include members from several species (see instant specification, page 18, last paragraph). In particular, the instant specification teaches that NOV4 has high homology (83% identity, and 85% similarity) to a mouse Kv channel protein AAC23664, and a human Kv channel protein P22001 (69% identity, and 81% similarity). At the time of filing of the instant application, the mouse protein AAC23664 had been shown to express in heart and skeletal muscle with high expression levels. See Kalman *et al.*, J. Biol. Chem. 273:5851-5857 (1998) (Exhibit 4 in Applicants' June 16 Amendment).

6. The instant specification at, *e.g.*, page 89, line 23, to page 96, line 2, describes screening assays that can be used to identify molecules that modulate the functions of the polypeptides of the invention, one of which being the polypeptide having the amino acid sequence of SEQ ID NO: 8. As discussed in Applicants' June 16 Amendment, methods of identifying molecules that can modulate functions of an identified ion-channel, such as a potassium channel, were also well-known and routine in the art at the time of the application was filed (see *e.g.*, Gonzalez et al., Drug Discov. Today 4(9):431-439 (1999) and U.S. Pat. No. 6,087,488, which were attached as Exhibits 2 and 3 in the June 16 Amendment, respectively). Thus, a person skilled in the art can readily perform a cell-based assay, as taught in the specification combined with the well

known knowledge in the art, to identify molecules that can modulate the functions of the potassium channel of the invention. Such modulators are likely useful as therapies for the treatment of disorders such as diabetes or neuromuscular disorders.

7. The instant specification also teaches how to detect a claimed nucleic acid in a biological sample (see specification, page 98, line 6, to page 100, line 16). At the time of the filing of the application, methods for detecting a nucleic acid in a biological sample, such as real time quantitative polymerase chain reaction ("RTQ-PCR"), were well known. The research group at CuraGen has performed studies evaluating the quantitative expression of the claimed nucleic acids encoding the polypeptide of SEQ ID NO: 8 in tissue culture cells and in isolated normal and pathological human tissue (see Exhibit 1 attached hereto).

8. As shown in Exhibit 1, the quantitative expression of the nucleic acid encoding the polypeptide of SEQ ID NO: 8 (referred to as the "CG53216-01") was examined using RTQ-PCR in isolated human tissues (both normal and pathological). The experimental conditions used to perform the RTQ-PCR are described in Exhibit 1. Table 1 indicates the specific oligonucleotide primers used in the experiment.

9. The result of the RTQ-PCR experiment clearly shows that CG53216-01 is highly expressed in skeletal muscle, consistent with the observations for the homologous mouse Kcna 7 gene. Thus, CG53216-01 (NOV4) can be used to differentiate skeletal muscle from other tissue types. For example, it can be used to differentiate a lung metastasized cancer that is skeletal muscle origin from the normal lung tissues or cancers that is lung tissue (or non-skeletal muscle tissue) origin, or vice versa.

10. In view of the discussion above, it is my opinion that a person skilled in the art can use the claimed nucleic acids as asserted in the specification without undue experimentation.

11. I hereby declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are

believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: Jan 21, 2005

Daniel Rieger

Dr. Daniel Rieger

Appendix 1

Curriculum Vitae

Daniel K. Rieger, Ph.D.

EXPERIENCE:

6/2003-present **Patent Scientist,** CuraGen Corporation, New Haven, CT
Intellectual Property Department

- Conduct IP analyses and solicit scientists' opinions to guide prosecution decisions, such as restriction/election, National Phase, pursue/drop
- Conduct freedom to operate analyses to facilitate collaborative small molecule pipeline decisions; present results to collaborator
- Draft provisional and non-provisional patent applications; claims; sequence listings
- Draft responses, amendments, restrictions/elections
- Inflammatory, metabolic, central nervous system disease programs
- Small molecule, protein and antibody drug targets
- Technology cases: expression profiling, nucleic acid amplification, genetic association tests
- Attended PLI's Patent Bar Review course

2001-03 **Research Scientist,** CuraGen Corporation, New Haven, CT
SNPCalling/Genetics & Gene Mining Group; Department of Drug Discovery
Field: Genetic association studies

- Oversaw SNP discovery, SNP validation and population genotyping
- Guided improvements and expansion of genetics software tools
- Responsible for interaction with genotyping vendors
- Collaborated with vendors in QC and troubleshooting of SNP assays
- Selected candidate disease genes by integration of proprietary and literature data
- Analyzed association test data provided by statisticians
- Communicated genetic data to collaborators
- Negotiated with prospective collaborators
- Performed homology-based mining of novel genes

1999-01 **Postdoctoral Fellow,** Harvard Medical School, Boston, MA
Department of Cell Biology (Advisor: Bjorn R. Olsen)
Topic: Genetic control of epithelial polarity during embryonic development

- Identified polymorphisms and mutations by PCR, SSCP, sequencing and Southern Blotting
- Physical mapping with YAC and BAC clones
- Identified a mutation in a mouse gene (Pitx3) that is required for maintenance of epithelial polarity during embryonic development
- Identified polymorphisms and mutations by PCR, SSCP, sequencing and Southern blotting
- Used immunohistology to study the function of the mutated gene
- PCR optimization, multiplex PCR and RTQ-PCR, Northern blotting

1992-98 **Graduate Research Assistant,** Wayne State University, Detroit, MI
Center for Molecular Medicine and Genetics (Advisor: Craig N. Giroux)
Topic: Regulation of recombination, chromosome structure, and cell division in yeast meiosis

- Created a system for gene expression in yeast based on a heat sensitive suppressor tRNA
- Developed a high resolution chromosome spreading technique for yeast
- Used electron microscopy and light microscopy in combination with digital imaging
- Used this technique to demonstrate that chromatin condensation precedes, and is independent from, the initiation of meiotic recombination
- Showed that meiotic recombination is initiated in chromatin loops
- Analyzed protein-protein interactions using yeast two-hybrid system and colocalization
- Utilized knockouts, suppressors and localization to study gene function and interactions in yeast
- Instructed students and technicians in experimental design and troubleshooting

1991-92 **Research Internship,** Wayne State University, Detroit, MI
Department of Molecular Biology and Genetics (Advisor: Leon Carlock)
Topic: Genetic and molecular analysis of Huntington disease

- Characterized a deletion in the promoter region of a Huntington disease candidate gene
- Studied protein-DNA interactions by DNAse I footprinting and gel shift assay
- Used a drug-induced rat model of Huntington disease to show that the candidate gene was coordinately induced with primary response genes during neurodegeneration

EDUCATION:

1999 **Ph.D.**, Molecular Biology and Genetics
Wayne State University School of Medicine, Detroit, Michigan
- Ph.D. thesis: Molecular analysis of an early meiotic decision point in yeast

1991 **Vordiplom** (B.S.), Biology
University of Freiburg, Germany

HONORS AND AWARDS:

- Postdoctoral Fellowship, NIH/NEI, Individual National Research Service Award, 2000-2001
- Postdoctoral Fellowship, NIH/NIDCR, Training Grant in Biomaterials,
Harvard School of Dental Medicine/Massachusetts Institute of Technology, 1999
- Fulbright Travel Grant (Fulbright Commission, Bonn, Germany), 1991-92
- T.C. Rumble Fellowship, 1993-94
- Graduate-Professional Scholarship, 1994-95
- Graduate Research Assistantships, 1992-93 and 1994-98
- T.C. Rumble Foreign Exchange Fellowship from the University of Freiburg, Germany, and Wayne State University, Detroit, 1991-92
- Award for outstanding service, Bundeswehr (German Army), 1988

PUBLICATIONS:

ARTICLES:

Rieger, D.K., E. Reichenberger, W. McLean, A. Sidow, and Olsen, B.R. (2001). A double-deletion mutation in the *Pitx3* gene causes arrested lens development in aphakia mice. Genomics *72*: 61-72.

ABSTRACTS:

Rieger, D.K. and Giroux C.N. (1997). Molecular basis of an early meiotic decision point. Mol Biol Cell 8 (S): 1556.

Rieger, D., Vo, T., Walker P. and Carlock, L. (1992). Molecular characterization of the Huntington disease animal model. Am J Hum Genet 51: A134.

Exhibit 1. Quantitative expression analysis of CG53216-01 in various cells and tissues

The quantitative expression of CG53216-01 was assessed using microtiter plates containing RNA samples from a variety of normal and pathology-derived cells, cell lines and tissues using real time quantitative PCR (RTQ-PCR) performed on an Applied Biosystems (Foster City, CA) ABI PRISM® 7700 or an ABI PRISM® 7900 HT Sequence Detection System.

RNA integrity of all samples was determined by visual assessment of agarose gel electropherograms using 28S and 18S ribosomal RNA staining intensity ratio as a guide (2:1 to 2.5:1 28s:18s) and the absence of low molecular weight RNAs (degradation products). Control samples to detect genomic DNA contamination included RTQ-PCR reactions run in the absence of reverse transcriptase using probe and primer sets designed to amplify across the span of a single exon.

RNA samples were normalized in reference to nucleic acids encoding constitutively expressed genes (i.e., β-actin and GAPDH). Alternatively, non-normalized RNA samples were converted to single strand cDNA (sscDNA) using Superscript II (Invitrogen Corporation, Carlsbad, CA, Catalog No. 18064-147) and random hexamers according to the manufacturer's instructions. Reactions containing up to 10 μg of total RNA in a volume of 20 μl or were scaled up to contain 50 μg of total RNA in a volume of 100 μl and were incubated for 60 minutes at 42°C. sscDNA samples were then normalized in reference to nucleic acids as described above.

Probes and primers were designed according to Applied Biosystems *Primer Express* Software package (version I for Apple Computer's Macintosh Power PC) or a similar algorithm using the target sequence as input. Default reaction condition settings and the following parameters were set before selecting primers: 250 nM primer concentration; 58°-60° C primer melting temperature (Tm) range; 59° C primer optimal Tm; 2° C maximum primer difference (if probe does not have 5' G, probe Tm must be 10° C greater than primer Tm; and 75 bp to 100 bp amplicon size. The selected probes and primers were synthesized by Synthegen (Houston, TX). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: 900 nM forward and reverse primers, and 200nM probe.

Normalized RNA was spotted in individual wells of a 96 or 384-well PCR plate (Applied Biosystems, Foster City, CA). PCR cocktails included a single gene-specific probe and primers set or two multiplexed probe and primers sets. PCR reactions were done using TaqMan® One-Step RT-PCR Master Mix (Applied Biosystems, Catalog No. 4313803) following manufacturer's instructions. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles: 95° C 10 min, then 40 cycles at 95° C for 15 seconds, followed by 60° C for 1 minute. Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) and plotted using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression was the reciprocal of the RNA difference multiplied by 100. CT values below 28 indicate high expression, between 28 and 32 indicate moderate expression, between 32 and 35 indicate low expression and above 35 reflect levels of expression that were too low to be measured reliably.

Normalized sscDNA was analyzed by RTQ-PCR using 1X TaqMan® Universal Master mix (Applied Biosystems; catalog No. 4324020), following the manufacturer's instructions. PCR amplification and analysis were done as described above.

Panels 1.1 and 1.2

Panels 1.1 and 1.2 included 2 control wells (genomic DNA control and chemistry control) and 94 wells of cDNA samples from cultured cell lines and primary normal tissues. Cell lines were derived from carcinomas (ca) including: lung, small cell (s cell var), non small cell (non-s or non-sm); breast; melanoma; colon; prostate; glioma (glio), astrocytoma (astro) and neuroblastoma (neuro); squamous cell (squam); ovarian; liver; renal; gastric and pancreatic from the American Type Culture Collection (ATCC, Bethesda, MD). Normal tissues were obtained from individual adults or fetuses and included: adult and fetal skeletal muscle, adult and fetal heart, adult and fetal kidney, adult and fetal liver, adult and fetal lung, brain, spleen, bone marrow, lymph node, pancreas, salivary gland, pituitary gland, adrenal gland, spinal cord, thymus, stomach, small intestine, colon, bladder, trachea, breast, ovary, uterus, placenta, prostate, testis and adipose. The following abbreviations are used in reporting the results: metastasis (met); pleural effusion (pl. eff or pl effusion) and * indicates established from metastasis.

General screening panel v1.7

Panel 1.7 was as described for Panels 1.1 and 1.2 above except that normal tissue samples were pooled from 2 to 5 different adults or fetuses.

Panels 2D

Panel 2D included 2 control wells and 94 wells containing RNA or cDNA from human surgical specimens procured through the National Cancer Institute's Cooperative Human Tissue Network (CHTN) or the National Disease Research Initiative (NDRI), Ardais (Lexington, MA) or Clinomics BioSciences (Frederick, MD). Tissues included human malignancies and in some cases matched adjacent normal tissue (NAT). Information regarding histopathological assessment of tumor differentiation grade as well as the clinical stage of the patient from which samples were obtained was generally available. Normal tissue RNA and cDNA samples were purchased from various commercial sources such as Clontech (Palo Alto, CA), Research Genetics and Invitrogen (Carlsbad, CA).

A. CG53216-01: AC008687_A, ORF start=38, ORF stop=1714, frame=2; 1747 bp.

Expression of gene CG53216-01 was assessed using the primer-probe set Ag512, described in Table AA. Results of the RTQ-PCR runs are shown in Tables AB, AC, AD and AE.

Table AA. Probe Name Ag512

Primers	Sequences	Length	Start Position	SEQ ID No

Forward	5'-ctgggcctcctcatcttttttc-3'	21	1280	
Probe	TET-5'-ttcatcggtgtggtcctcttttccagc-3'-TAMRA	27	1304	
Reverse	5'-cggtcaacttcggcaaagtag-3'	21	1336	

Table AB. General_screening_panel_v1.7

Column A - Rel. Exp.(%) Ag512, Run 318348641			
Tissue Name	**A**	**Tissue Name**	**A**
Adipose	4.7	Gastric ca. (liver met.) NCI-N87	0.1
HUVEC	0.3	Stomach	0.0
Melanoma* Hs688(A).T	0.0	Colon ca. SW-948	0.2
Melanoma* Hs688(B).T	17.9	Colon ca. SW480	0.0
Melanoma (met) SK-MEL-5	0.2	Colon ca. (SW480 met) SW620	0.8
Testis	0.1	Colon ca. HT29	0.0
Prostate ca. (bone met) PC-3	0.1	Colon ca. HCT-116	3.4
Prostate ca. DU145	0.5	Colon cancer tissue	0.0
Prostate pool	0.5	Colon ca. SW1116	0.7
Uterus pool	0.0	Colon ca. Colo-205	0.0
Ovarian ca. OVCAR-3	0.1	Colon ca. SW-48	0.0
Ovarian ca. (ascites) SK-OV-3	0.7	Colon	0.0
Ovarian ca. OVCAR-4	2.4	Small Intestine	0.1
Ovarian ca. OVCAR-5	1.7	Fetal Heart	0.1
Ovarian ca. IGROV-1	0.7	Heart	0.2
Ovarian ca. OVCAR-8	1.9	Lymph Node Pool	0.0
Ovary	0.2	Lymph Node pool 2	0.4
Breast ca. MCF-7	0.1	Fetal Skeletal Muscle	6.2
Breast ca. MDA-MB-231	5.8	Skeletal Muscle pool	5.4
Breast ca. BT 549	0.6	Skeletal Muscle	**100.0**
Breast ca. T47D	0.5	Spleen	0.1
113452 mammary gland	0.1	Thymus	0.1
Trachea	1.6	CNS cancer (glio/astro) SF-268	0.2
Lung	0.4	CNS cancer (glio/astro) T98G	1.7
Fetal Lung	0.1	CNS cancer (neuro;met) SK-N-AS	0.0
Lung ca. NCI-N417	0.0	CNS cancer (astro) SF-539	9.0
Lung ca. LX-1	0.2	CNS cancer (astro) SNB-75	4.1
Lung ca. NCI-H146	0.0	CNS cancer (glio) SNB-19	2.1
Lung ca. SHP-77	0.0	CNS cancer (glio) SF-295	1.6
Lung ca. NCI-H23	4.0	Brain (Amygdala)	0.1

Lung ca. NCI-H460	0.5	Brain (Cerebellum)	0.1
Lung ca. HOP-62	2.0	Brain (Fetal)	0.1
Lung ca. NCI-H522	1.6	Brain (Hippocampus)	0.0
Lung ca. DMS-114	1.0	Cerebral Cortex pool	0.0
Liver	0.0	Brain (Substantia nigra)	0.0
Fetal Liver	0.0	Brain (Thalamus)	0.0
Kidney pool	0.1	Brain (Whole)	0.0
Fetal Kidney	0.1	Spinal Cord	0.1
Renal ca. 786-0	3.9	Adrenal Gland	0.4
Renal ca. A498	3.9	Pituitary Gland	0.3
Renal ca. ACHN	3.6	Salivary Gland	0.2
Renal ca. UO-31	1.0	Thyroid	1.4
Renal ca. TK-10	3.5	Pancreatic ca. PANC-1	0.2
Bladder	0.3	Pancreas pool	0.0

Table AC. Panel 1.1

Column A - Rel. Exp.(%) Ag512, Run 109023488			
Tissue Name	**A**	**Tissue Name**	**A**
Adrenal gland	0.0	Renal ca. UO-31	0.1
Bladder	0.0	Renal ca. RXF 393	0.2
Brain (amygdala)	0.0	Liver	0.0
Brain (cerebellum)	0.0	Liver (fetal)	0.0
Brain (hippocampus)	0.0	Liver ca. (hepatoblast) HepG2	0.0
Brain (substantia nigra)	0.0	Lung	0.0
Brain (thalamus)	0.0	Lung (fetal)	0.0
Brain (hypothalamus)	0.0	Lung ca. (non-s.cell) HOP-62	0.6
Brain (fetal)	0.0	Lung ca. (large cell)NCI-H460	0.0
Brain (whole)	0.0	Lung ca. (non-s.cell) NCI-H23	0.4
glio/astro U-118-MG	0.2	Lung ca. (non-s.cl) NCI-H522	0.7
astrocytoma SF-539	0.4	Lung ca. (non-sm. cell) A549	0.3
astrocytoma SNB-75	0.1	Lung ca. (s.cell var.) SHP-77	0.0
astrocytoma SW1783	0.3	Lung ca. (small cell) LX-1	0.2
glioma U251	0.2	Lung ca. (small cell) NCI-H69	0.3
glioma SF-295	1.0	Lung ca. (squam.) SW 900	0.5
glioma SNB-19	0.2	Lung ca. (squam.) NCI-H596	0.0
glio/astro U87-MG	1.6	Lymph node	0.0
neuro*; met SK-N-AS	0.0	Spleen	0.0

Mammary gland	0.2	Thymus	0.0
Breast ca. BT-549	0.0	Ovary	0.0
Breast ca. MDA-N	0.0	Ovarian ca. IGROV-1	0.3
Breast ca.* (pl. ef) T47D	0.2	Ovarian ca. OVCAR-3	0.0
Breast ca.* (pl.ef) MCF-7	0.0	Ovarian ca. OVCAR-4	0.1
Breast ca.* (pl.ef) MDA-MB-231	0.1	Ovarian ca. OVCAR-5	1.4
Small intestine	0.0	Ovarian ca. OVCAR-8	0.2
Colon (ascending)	0.0	Ovarian ca. (ascites) SK-OV-3	1.3
Colon ca. HT29	0.0	Pancreas	0.0
Colon ca. CaCo-2	0.3	Pancreatic ca. CAPAN 2	0.4
Colon ca. HCT-15	0.1	Pituitary gland	0.4
Colon ca. HCT-116	0.2	Placenta	1.6
Colon ca. HCC-2998	0.7	Prostate	0.2
Colon ca. SW480	0.1	Prostate ca.* (bone met) PC-3	0.2
Colon ca.* SW620 (SW480 met)	0.0	Salivary gland	0.2
Stomach	0.0	Trachea	0.1
Gastric ca. (liver met) NCI-N87	0.8	Spinal cord	0.0
Heart	0.3	Testis	0.2
Myometrium	6.5	Thyroid	0.7
Skeletal muscle	**100.0**	Uterus	0.0
Endothelial cells	0.0	Melanoma M14	0.0
Heart (Fetal)	0.0	Melanoma LOX IMVI	0.2
Kidney	0.0	Melanoma UACC-62	0.0
Kidney (fetal)	0.1	Melanoma SK-MEL-28	0.0
Renal ca. 786-0	0.4	Melanoma* (met) SK-MEL-5	0.0
Renal ca. A498	0.9	Melanoma Hs688(A).T	0.2
Renal ca. ACHN	0.6	Melanoma* (met) Hs688(B).T	0.3
Renal ca. TK-10	0.8		

Table AD. Panel 1.2

Column A - Rel. Exp.(%) Ag512, Run 117657368			
Tissue Name	**A**	**Tissue Name**	**A**
Endothelial cells	0.0	Renal ca. 786-0	0.2
Heart (Fetal)	0.0	Renal ca. A498	1.0
Pancreas	0.1	Renal ca. RXF 393	0.2
Pancreatic ca. CAPAN 2	0.6	Renal ca. ACHN	0.9
Adrenal gland	0.0	Renal ca. UO-31	0.1

Thyroid	1.3	Renal ca. TK-10	0.6
Salivary gland	0.7	Liver	0.0
Pituitary gland	0.5	Liver (fetal)	0.0
Brain (fetal)	0.0	Liver ca. (hepatoblast) HepG2	0.0
Brain (whole)	0.0	Lung	0.0
Brain (amygdala)	0.0	Lung (fetal)	0.0
Brain (cerebellum)	0.0	Lung ca. (small cell) LX-1	0.1
Brain (hippocampus)	0.0	Lung ca. (small cell) NCI-H69	0.1
Brain (thalamus)	0.0	Lung ca. (s.cell var.) SHP-77	0.0
Cerebral Cortex	0.0	Lung ca. (large cell)NCI-H460	0.1
Spinal cord	0.0	Lung ca. (non-sm. cell) A549	0.2
glio/astro U87-MG	1.8	Lung ca. (non-s.cell) NCI-H23	0.4
glio/astro U-118-MG	0.4	Lung ca. (non-s.cell) HOP-62	0.3
astrocytoma SW1783	0.5	Lung ca. (non-s.cl) NCI-H522	0.9
neuro*; met SK-N-AS	0.0	Lung ca. (squam.) SW 900	0.8
astrocytoma SF-539	0.5	Lung ca. (squam.) NCI-H596	0.1
astrocytoma SNB-75	0.1	Mammary gland	0.2
glioma SNB-19	0.3	Breast ca.* (pl.ef) MCF-7	0.0
glioma U251	0.3	Breast ca.* (pl.ef) MDA-MB-231	0.1
glioma SF-295	0.8	Breast ca.* (pl. ef) T47D	0.2
Heart	0.4	Breast ca. BT-549	0.0
Skeletal muscle	**100.0**	Breast ca. MDA-N	0.0
Bone marrow	0.0	Ovary	0.0
Thymus	0.0	Ovarian ca. OVCAR-3	0.1
Spleen	0.0	Ovarian ca. OVCAR-4	0.2
Lymph node	0.0	Ovarian ca. OVCAR-5	1.1
Colorectal	0.0	Ovarian ca. OVCAR-8	0.2
Stomach	0.0	Ovarian ca. IGROV-1	0.2
Small intestine	0.0	Ovarian ca. (ascites) SK-OV-3	2.0
Colon ca. SW480	0.0	Uterus	0.0
Colon ca.* SW620 (SW480 met)	0.0	Placenta	2.7
Colon ca. HT29	0.0	Prostate	0.3
Colon ca. HCT-116	0.2	Prostate ca.* (bone met) PC-3	0.2
Colon ca. CaCo-2	0.4	Testis	0.5
CC Well to Mod Diff (ODO3866)	0.0	Melanoma Hs688(A).T	0.3
Colon ca. HCC-2998	0.6	Melanoma* (met) Hs688(B).T	0.4
Gastric ca. (liver met) NCI-N87	1.1	Melanoma UACC-62	0.0
Bladder	0.1	Melanoma M14	0.1

Trachea	0.1	Melanoma LOX IMVI	0.3
Kidney	0.0	Melanoma* (met) SK-MEL-5	0.0
Kidney (fetal)	0.1		

Table AE. Panel 2D

Column A - Rel. Exp.(%) Ag512, Run 145928352			
Tissue Name	**A**	**Tissue Name**	**A**
Normal Colon	5.8	Kidney Margin 8120608	1.0
CC Well to Mod Diff (ODO3866)	0.0	Kidney Cancer 8120613	0.0
CC Margin (ODO3866)	3.2	Kidney Margin 8120614	0.5
CC Gr.2 rectosigmoid (ODO3868)	1.9	Kidney Cancer 9010320	1.6
CC Margin (ODO3868)	0.0	Kidney Margin 9010321	0.0
CC Mod Diff (ODO3920)	0.2	Normal Uterus	0.0
CC Margin (ODO3920)	2.5	Uterine Cancer 064011	3.1
CC Gr.2 ascend colon (ODO3921)	0.8	Normal Thyroid	21.6
CC Margin (ODO3921)	0.9	Thyroid Cancer	2.1
CC from Partial Hepatectomy (ODO4309) Mets	0.0	Thyroid Cancer A302152	0.0
Liver Margin (ODO4309)	2.1	Thyroid Margin A302153	0.0
Colon mets to lung (OD04451-01)	2.0	Normal Breast	5.0
Lung Margin (OD04451-02)	2.1	Breast Cancer	1.2
Normal Prostate 6546-1	6.3	Breast Cancer (OD04590-01)	1.6
Prostate Cancer (OD04410)	0.9	Breast Cancer Mets (OD04590-03)	0.0
Prostate Margin (OD04410)	12.6	Breast Cancer Metastasis	0.6
Prostate Cancer (OD04720-01)	10.7	Breast Cancer	2.6
Prostate Margin (OD04720-02)	11.1	Breast Cancer	2.0
Normal Lung	0.2	Breast Cancer 9100266	2.6
Lung Met to Muscle (ODO4286)	4.4	Breast Margin 9100265	2.7
Muscle Margin (ODO4286)	**100.0**	Breast Cancer A209073	5.0
Lung Malignant Cancer (OD03126)	3.0	Breast Margin A209073	4.4
Lung Margin (OD03126)	0.9	Normal Liver	0.0
Lung Cancer (OD04404)	3.0	Liver Cancer	0.0
Lung Margin (OD04404)	1.8	Liver Cancer 1025	1.4
Lung Cancer (OD04565)	6.6	Liver Cancer 1026	0.6
Lung Margin (OD04565)	0.0	Liver Cancer 6004-T	1.8
Lung Cancer (OD04237-01)	3.5	Liver Tissue 6004-N	0.4
Lung Margin (OD04237-02)	0.0	Liver Cancer 6005-T	0.0
Ocular Mel Met to Liver (ODO4310)	3.1	Liver Tissue 6005-N	0.0

Liver Margin (ODO4310)	0.6	Normal Bladder	2.8
Melanoma Metastasis	0.0	Bladder Cancer	1.3
Lung Margin (OD04321)	0.5	Bladder Cancer	4.2
Normal Kidney	1.3	Bladder Cancer (OD04718-01)	14.7
Kidney Ca, Nuclear grade 2 (OD04338)	0.0	Bladder Normal Adjacent (OD04718-03)	0.0
Kidney Margin (OD04338)	0.0	Normal Ovary	0.5
Kidney Ca Nuclear grade 1/2 (OD04339)	1.3	Ovarian Cancer	0.5
Kidney Margin (OD04339)	0.0	Ovarian Cancer (OD04768-07)	0.0
Kidney Ca, Clear cell type (OD04340)	3.3	Ovary Margin (OD04768-08)	0.0
Kidney Margin (OD04340)	4.6	Normal Stomach	0.0
Kidney Ca, Nuclear grade 3 (OD04348)	8.8	Gastric Cancer 9060358	0.0
Kidney Margin (OD04348)	0.0	Stomach Margin 9060359	1.3
Kidney Cancer (OD04622-01)	0.6	Gastric Cancer 9060395	2.0
Kidney Margin (OD04622-03)	0.0	Stomach Margin 9060394	0.4
Kidney Cancer (OD04450-01)	0.0	Gastric Cancer 9060397	1.2
Kidney Margin (OD04450-03)	3.0	Stomach Margin 9060396	0.0
Kidney Cancer 8120607	1.9	Gastric Cancer 064005	3.0

Panel 1.1 Summary: Ag512 Expression of this gene is highest in skeletal muscle. The CG53216-01 gene encodes a protein with homology to the mouse Shaker-related voltage-gated potassium channel, Kv1.7 (Kcna7). The mouse Kcna7 gene is expressed in skeletal muscle, heart and kidney, consistent with the observations in this experiment. In situ hybridization of the mouse gene shows that it is expressed in pancreatic islet cells. Interestingly, the human KCNA gene was reported to map to human chromosome 19q13.3, a region that has been suggested to contain a diabetic susceptibility locus. Therefore, therapeutic modulation of the activity of this gene may prove useful in the treatment of endocrine/metabolically related diseases, such as obesity and diabetes.

1: Kalman K, Nguyen A, Tseng-Crank J, Dukes ID, Chandy G, Hustad CM, Copeland NG, Jenkins NA, Mohrenweiser H, Brandriff B, Cahalan M, Gutman GA, Chandy KG. Genomic organization, chromosomal localization, tissue distribution, and biophysical characterization of a novel mammalian Shaker-related voltage-gated potassium channel, Kv1.7. J Biol Chem 1998 Mar 6;273(10):5851-7

Panel 1.2 Summary: Ag512 Expression of this gene is highest in skeletal muscle.

Panel 2D Summary: Ag512 Expression of this gene is highest in normal skeletal muscle adjacent to a lung tumor metastasis (CT = 29.2). These results are consistent with what is observed in Panels 1.1 and 1.2.